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Salt Induced Variation on the Bioactive Components of Fermented Oil Bean (*Pentaclethra macrophylla Benth*) Seeds

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ABSTRACT

Background and Objective: Processing of *Pentaclethra macrophylla Benth* seeds into "Ugba" delicacy not only reduces the anti-nutrient concentrations but also introduces some unique compounds that improves its nutritional potentials and enhances its acceptability and palatability.

Methodology: A quantity of 2.0 kg of raw sample was boiled in water at 100 °C for 12 hrs, the testa was dehulled and 10 g of edible salt was added to the sliced cotyledons. The mixture was boiled for 3 hrs and allowed to stand for 24 hrs at room temperature. The sliced cotyledons were filtered and divided into three parts of 0.5 kg each. One part was ground immediately into a smooth paste to give the "cooked-salted unfermented sample". The 2nd and 3rd parts were seeded with 0.2 g each of three days fermented *P. macrophylla Benth* seed and allowed to ferment. The second part was ground after 48 hours while the third part was ground after 96 hours to give"cooked-salted 2 days fermented" and "cooked-salted 4 days fermented" samples respectively.

Results: Oleic acid was the highest volatile component observed via GC-MS analysis of cooked salted unfermented sample with a total percentage concentration of 94.909, while 9,12-Octadecadienoic acid (Z,Z)- (Linoliec acid) was the highest bioactive component observed in cooked salted two days fermented and cooked salted four days fermented samples with total percentage concentrations 96.807 and 55.598 respectively.

Conclusion: This study shows that the product of *P. macrophylla Benth* fermentation is a factor of both fermentation conditions and predominant fermenting organism favoured by such conditions.

Keywords: Microorganism, fermentation, bioactive components, anti-nutrients, therapeutic properties.

The African oil bean tree is a leguminous plant with oil bearing seeds. It is the sole member of the genus-*Pentaclethra* occurring naturally in the humid lowlands of West Africa and is valued by peasant farmers in Southeast Nigeria for its soil improvement properties[1]. The seeds are prepared, fermented and eaten as oil bean salad or used as soup condiment in Nigeria. Though the unfermented raw oil bean seed is a potential source of protein and fatty acids[2], it is suspected to contain a number of anti-nutritional and/or toxic compounds such as saponins, alkaloids, sterols, glycosides, and growth depressant caffeolyputrescine[3]. Reports on its proximate analysis shows moisture (11.87%), crude protein (36.2-43.89%), carbohydrate (14.79-18%), saturated fatty acids (12%), crude fiber (2.50%), ash (2.95%)[4]. He also reported the presence of 20 essential amino acids and other minerals such as calcium, iron, sulphur, phosphorus[4]. Therapeutic reports have shown that oil bean seeds have the potential to prevent and cure different diseases such as high blood pressure, heart disease, obesity, hypertension, diarrhea, epilepsy, malnutrition, stomach disorder, microbes, iron deficiency, eye problem and insomnia[5]. They also reported oil bean seed as an anti-microbial wound healing agent, that boosts immune system and inhibit

the growth *of Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia coli* among others[5]. Studies have also show that about 60% of people eat oil bean seed because of its delicacy while 40% eat it because of its multi-therapy capabilities[6]. Irrespective of the therapeutic properties, raw African oil bean seeds have been reported to be high in tannins, phytates, oxalates and lectins as well as high levels of trypsin inhibitor activity[2]. However, processing the seed into "ugba" via cooking and fermentation significantly reduces the levels of the anti-nutritional factors[7] and improves the acceptability, palatability, digestibility and durability of the produce[8]. This present research studies the effect of cooking and salting on the bioactive components of fermented oil bean seed.

MATERIALS AND METHODS

Collection and preparation of sample

Fresh seeds of *P. macrophylla Benth* were purchased from Rumuokoro Market in Obio/Akpor Local Government Area of Rivers State and were taken to University of Port Harcourt, Rivers State, Nigeria. A quantity of 2.0 kg of raw sample was boiled in water at 100 ˚C for 12 hrs, the testa was dehulled and the cotyledons were sliced using a sterilized knife. A quantity of 10 g of edible salt was added to the sliced cotyledons. The mixture was boiled again for 3 hrs and allowed to stand at room temperature. After 24 hrs, the sliced cotyledons were filtered and divided into three parts of 0.5 kg each. One part was ground immediately into a smooth paste using Thomas Scientific, (Model 4) Wiley's mill to give the "cooked-salted unfermented sample" and the resultant paste was immediately analysed. The second and third parts were seeded with 0.2 g each of three days fermented *P. macrophylla Benth* seed and allowed to ferment. The second part was ground after 48 hours to give"cooked-salted 2 days fermented" sample, the resultant paste was immediately analysed, while the third part was ground after 96 hours to give "cooked-salted 4 days fermented" sample and the resultant paste was also analysed immediately.

GC-MS analysis of P. macrophylla Benth Oil

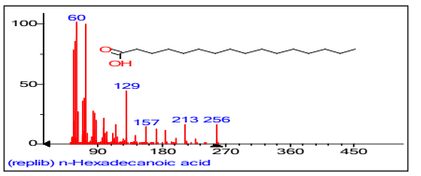
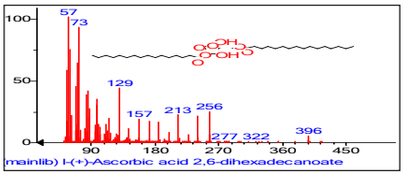
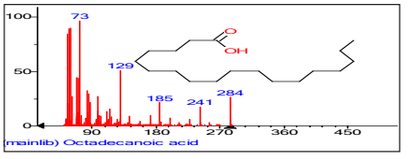
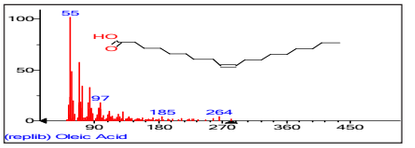
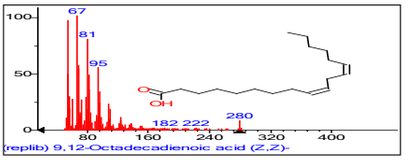
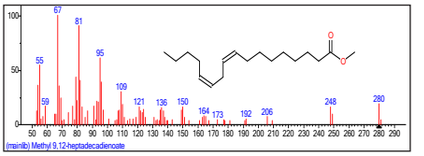
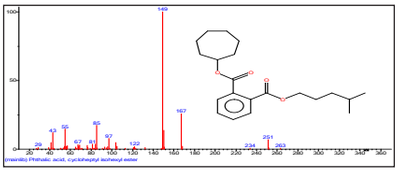
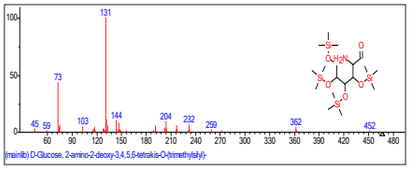
A hundred gram each of the ground samples of *P. macrophylla Benth* seed paste were separately added to 3 dm^3 of distilled water. The oil obtained by hydro-distillation was collected into hexane and the solution was concentrated by evaporation at room temperature. The oil was analysed using a combined gas chromatograph model HP 6890 and mass spectrometer model 5973 (AgilentTech.) fitted with a capillary column HP-5 MS (5% phenylmethylsiloxane) 30.0 m × 250μm × 0.25μm, using Helium as a carrier gas at initial column temperature 120°C for 5 minutes. Thereafter, the column temperature was increased at 5°C per minutes to 320°C and held for 5 minutes. Electron impact ionization for mass spectroscopy was done at ionization energy of 70eV. The oil was diluted with 98% hexane and 2μl of the diluted sample was automatically injected into AgilentTech model 5973 mass spectrometer. The constituent compounds were identified using the Chem-Office software attached to the MS library. The names molecular formula and molecular weights of the component oils were ascertained using the database of National Institute of Standard and Technology (NIST).

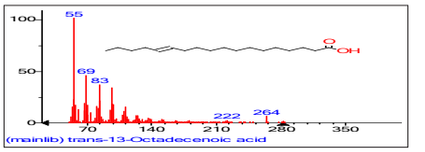
RESULTS AND DISCUSSION

The bioactive components observed in cooked salted unfermented, cooked salted two days fermented and cooked salted four days fermented *P. macrophylla Benth* seeds, their retention times, percentage concentration, molecular formula, molecular weight, spectra and structures are shown in Tables 1 - 3. Oleic acid was the highest volatile component observed in cooked salted unfermented *P. macrophylla Benth* seed, with a total percentage concentration of 94.909 and a mean retention time of 19.917 min., while 9,12-Octadecadienoic acid (Z,Z)- (Linoliec acid) was the highest volatile component observed in cooked salted two days fermented and cooked salted four days fermented seed, with total percentage concentrations 96.807 and 55.598 and mean retention times of 20.630 min. and 19.311 mins. respectively.

Discussion

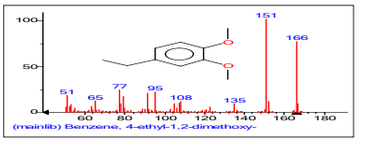
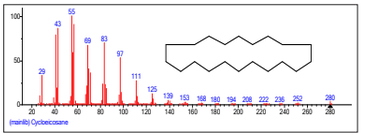
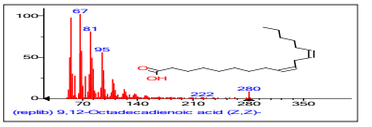
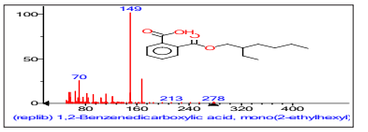
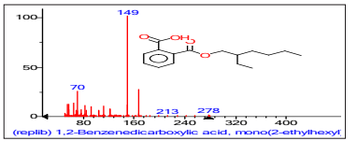
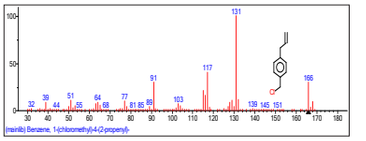
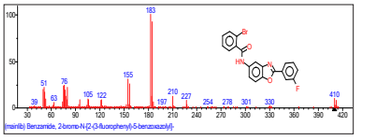
Table 1: Bioactive components of cooked salted unfermented *Pentaclethra macrophylla Benth* seed

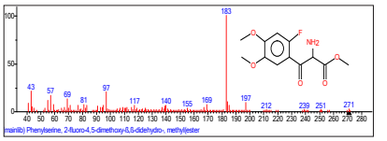
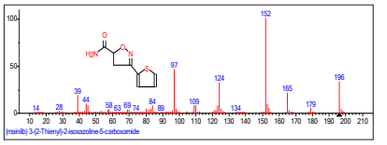
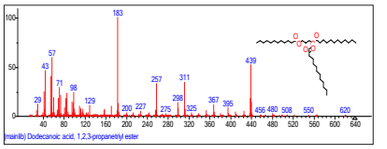
Sl. No.	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
1	n-Hexadecanoic acid	18.751[R7]	0.598[c7]	$C_{16}H_{32}O_2$	256.4241	
2	l-(+)-Ascorbic acid 2,6-dihexadecanoate	19.246[R2]	0.584[c2]	$C_{38}H_{68}O_8$	652.9417	
3	Octadecanoic acid	19.400	0.283	$C_{18}H_{36}O_2$	284.4772	
4	Oleic Acid	19.917[R3]	94.909[c3]	$C_{18}H_{34}O_2$	282.4614	
5	9,12-Octadecadienoic acid (Z,Z)-	21.464	0.031	$C_{19}H_{34}O_2$	294.4721	
6	Methyl 9,12-heptadecadienoate	21.777	0.220	$C_{18}H_{32}O_2$	280.4450	
7	Phthalic acid, cycloheptyl isohexyl ester	23.585	0.521	$C_{18}H_{24}O_4$	304.3807	
8	D-Galactose, 2-amino-2-deoxy-3,4,5,6-tetrakis-O-(trimethylsilyl)-	25.499	1.917	$C_{18}H_{45}NO_5Si_4$	419.8974	

9	trans-13-Octadecenoic acid	36.295	0.935	$C_{19}H_{36}O_2$	296.4879	

Note: *Superscripts R7, R2 and R3 are mean values of seven, two and three retention times respectively, while C7, C2 and C3 are sum of seven, two and three percentage concentrations respectively.*

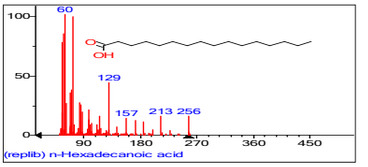
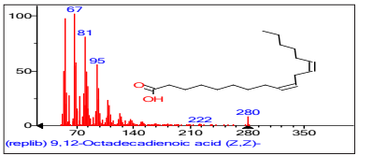
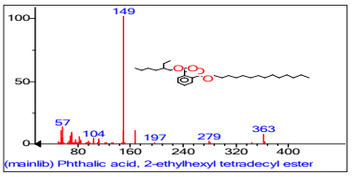
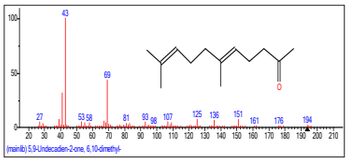
Table 2: Bioactive components of cooked salted two days fermented *Pentaclethra macrophylla Benth* seed

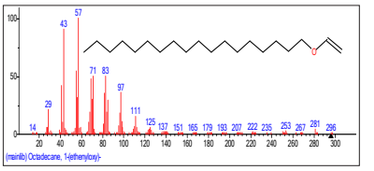
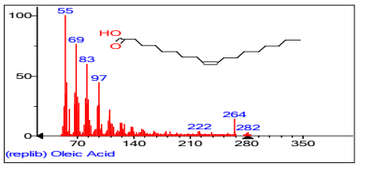
S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
1	Benzene, 4-ethyl-1,2-dimethoxy-	9.109	0.285	$C_{10}H_{14}O_2$	166.2170	
2	Cycloeicosane	18.485	0.064	$C_{20}H_{40}$	280.5316	
3	9,12-Octadecadienoic acid (Z,Z)-	20.630^{R5}	96.807^{c5}	$C_{18}H_{32}O_2$	280.4455	
4	1,2-Benzene dicarboxylic acid, mono (2-ethylhexyl) ester	23.474	0.999	$C_{16}H_{22}O_4$	278.3435	
4	1,2-Benzenedicarboxy lic acid, mono (2-ethylhexyl) ester	23.474	0.999	$C_{16}H_{22}O_4$	278.3435	
5	Benzene, 1-(chloromethyl)-4-(2-propenyl)-	25.321	0.088	$C_{10}H_{11}Cl$	166.6943	
6	Benzamide, 2-bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl]-	35.895	0.388	$C_{20}HN_2O_2$ BrF	467.0107	

7	Phenylserine, 2-fluoro-4,5-dimetho	35.927	0.188	$C_{12}H_2NO_5F$	259.1268	
8	3-(2-Thienyl)-4,5-dihydro-5-isoxazolemethanol	35.955	0.295	$C_8H_9NO_2S$	183.2276	
9	Dodecanoic acid, 1,2,3-propanetriyl ester	38.289^{R5}	0.886^{c5}	$C_{39}H_{74}O_6$	639.0013	

Note: *Superscripts R5, and C5 are mean values of five retention times and sum of five percentage concentrations respectively.*

Table 3: Bioactive components of cooked salted four days fermented *Pentaclethra macrophylla Benth* seed

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
1	n-Hexadecanoic acid	17.530^{R9}	9.463^{c9}	$C_{16}H_{32}O_2$	256.4241	
2	9,12-Octadecadienoic acid (Z,Z)-	19.311^{R13}	55.598^{c13}	$C_{18}H_{32}O_2$	280.4455	
3	Phthalic acid, 2-ethylhexyl tetradecyl ester	23.895	8.173	$C_{30}H_{50}O_4$	474.7156	
4	5,9-Undecadien-2-one, 6,10-dimethyl-	30.196	7.435	$C_{13}H_{22}O$	194.3132	

5	Octadecane, 1-(ethenyloxy)-	37.252	15.531	$C_{20}H_{40}O$	296.5310	
6	Oleic Acid	37.303	3.800	$C_{18}H_{34}O_2$	282.4614	

Note: *Superscripts R9 and R13 are mean values of nine and thirteen retention times, while C9 and C13 are sum of nine and thirteen percentage concentrations respectively.*

The result of this study showed pronounced variation in both the structures and concentration of the volatile components observed in *P. macrophylla Benth* seeds subjected to both salt processing and second cooking. This indicates that both second cooking and salting induces some notable alterations on the biochemical components of this delicacy. The high concentration of oleic acid observed after slicing and second cooking of *P. macrophylla Benth* seeds corroborates the work of Ohiri and Essien[9], where 55.204% of the total observed essential oil in cooked dehulled and sliced *P. macrophylla Benth* seeds was the E-methyl ester of 9-Octadecenoic acid (Oleic acid). This high concentration of oliec acid observed in this study (94.909%) indicates that second cooking (cooking after slicing) of this delicacy may be paramount in the release of this important essential oil. However, the presence of anti-nutrients may render this delicacy slightly unpalatable thereby affecting the consumption this essential nutrient. Though the high concentration of oleic acid in this delicacy maybe helpful in repealing attacks from insects during fermentation, as a omega 9 fatty acid, oleic acid has been reported to regulate the activities of adrenoreceptor signaling pathways which direct the α- and β-adrenoceptors, thereby regulating blood pressure[10]. Ruiz-Gutiérrez *et al.*[11], reported a reduction in blood pressure and an increase in high density lipoprotein (HDL) cholesterol in women after 4 weeks of consumption of high oleic acid diet. Oleic acid has also been reported to increase fat oxidation in muscles cells by increasing the expression of genes involved in β-oxidation[12]. The consumption of oleic acid rich diet not only reduces age-related changes in the brain's mitochondria[13], it also increases plasticity in infant males[14] Its ability to reduce obesity dependent inflammations is dependent on its inhibitory potentials on the inflammatory cytokine TNF-α which contributes to metabolic syndrome when produced by adipose cells[15,16]. The potential of oleic acid to protect cell membrane from radicals and other oxidative stressors is due to its ability to replace omega-3 and omega-6 fatty acid in the cell membrane and its increased resistance to oxidative damage[17].

The high concentration of 9,12-Octadecadienoic acid (linoleic acid) observed after two days of fermenting cooked and salted *P. macrophylla Benth* seeds (96.807%) shows a possible microbial enzymatic conversion of oleic acid to linoleic acid, which entails the introduction of a double bond at carbon 12 of the aliphatic chain. This conversion shows the presence of a non-conventional microorganism, whose presence and metabolic activities is been favoured by the alkaline environment generated due to the addition of NaCl (common salt) to the delicacy prior to its fermentation. Though microbial studies of *P. macrophylla Benth* seed fermentation identified *Bacillus* spp *(Bacillus subtilis*) as the main fermenting organisms[18], the presence and activities of other species such *B. pumilus, B. megaterium, B. lichenformis* were also been reported[18]. However, these organisms

may not be responsible for the conversion of oleic acid to linoleic acid as suspected in this study. Yuan and Bloch[19], reported that the high content of linoleate found in an edible lipid-rich yeast (*Torulopsis utilis*), is due to its ability to efficiently use oleate as a precursor for the synthesis of linoleate. Harris and James[20], also report the ability of a medicinal unicellular green algae (*Chlorella vulgaris*) to readily convert oleic acid to linoleic acid.

Linoleic acid is used in the biosynthesis of arachidonic acid, some prostaglandins, leukotrienes and thromboxane. As a polyunsaturated essential fatty acid, linoleic acid must be consumed for proper health. Though linoleic acid is found among the lipids of the cell membrane, its deficiency has been reported to cause skin scaling, hair loss[21], and poor wound healing in rats[22]. The decrease in the concentration of linoleic acid (55.598%) after four days of fermentation may be attributed to an enzymatic conversion of linoleic acid to either another metabolic intermediate or a product. This corroborates the work Harris and James[20], which reported linoleic acid as an intermediate compound in the enzymatic conversion of oleic acid to linolenic acid. This finding presents linoleic acid as an accumulated metabolic intermediate, whose high concentration may cause a feedback inhibition at a point in the metabolic pathway where a backward reaction can cause a saturation of the omega-6 double bond, thus leading to the regeneration of oleic acid. This may be responsible for the slight reintroduction of oleic acid (3.800%) into the delicacy after four days of fermentation.

CONCLUSION

An alteration in the pH of *P. macrophylla Benth* seeds which may have been achieved via addition of edible salt (NaCl) and cooking prior to fermentation induces a growth condition that may supress bacterial growth/ fermentation but enhances the growth/fermentation by other strains such as algae and fungi. This indicate that products of this fermented delicacy is a factor of the predominant fermenting organism and the fermentation condition.

SIGNIFICANCE STATEMENT

The findings from this study shows that the fermentation product of this delicacy is not limited to specific microbial species rather, it is determined by the fermentation condition and predominant fermenting organism.

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